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Evolution of Organelles and Eukaryotic Genomes

Separation of genes for chloroplast ribosomes in two genomes suggests principles of organelle biology.

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Some genetic determinants for chloroplast characters are transmitted from either parent, as would be expected from Mendel's rule that alleles segregate independently. In many, but not all plants, other plastid features are inherited only from the maternal parent in a pattern contrary to the principles of nuclear genetics. Extranuclear, or cytoplasmic, inheritance was recognized in eukaryotic green plants at the beginning of this century and is now a well-documented phenomenon [reviewed in (*1*)]. Some hereditary defects in yeast mitochondria are transmitted in a Mendelian manner. Others, such as lesions induced by acridine dyes, are not only unexpressed but are permanently lost after crossing with wild-type yeast; that is, the inheritance is uniparental and not Mendelian (*2*, *3*). These observations suggest (i) that plastids and mitochondria have their own systems of gene transmission and thus could be genetically autonomous and (ii) that nuclear genes directly or indirectly affect the health and survival of these organelles. Compartmentalization of functions in membrane-limited nuclei, mitochondria, and plastids is the hallmark of eukaryotic life, and understanding the genetic and metabolic interactions of organelles and the nuclear-cytoplasmic system a central problem of cell biology.

The development of molecular genetics brought the expectation that independent heredity should mean independent DNA as well as independent machinery for the production of RNA's and proteins. This led to a vigorous search for DNA, ribosomes, transfer RNA's (tRNA's), and related intermediaries in organelles (*4*). By means of techniques newly available in the 1960's, ribosomes were identified in chloroplasts and were shown to be different in size from those in the cytoplasm (*5*, *6*). Soon afterward DNA was detected in chloroplasts and was shown to be organized differently from that in nuclear chromosomes (*7*). Mitochondria also contain DNA and ribosomes (*8*). The characterization of DNA's, ribosomes, and other components of transcriptional and translational systems in plastids and mitochondria is still incomplete and is the subject of ongoing research.

A number of observations have led to the suggestion that the information storage and processing systems of organelles resemble those of bacteria and blue-green algae—that is, prokaryotes—more than they do the functionally equivalent elements of the nucleo-cytoplasmic system in the cells of which the organelles are parts. (i) The DNA's of mitochondria, plastids, and prokaryotes are similar when viewed in situ with the electron microscope; some features of their renaturation are also somewhat alike. (ii) Ribosomes of chloroplasts and mitochondria, like those of prokaryotes, tend to be smaller than those in the cytoplasm of eukaryotic cells. (iii) Many antibiotics which block protein synthesis by prokaryotic ribosomes affect plastid and mitochondrial ribosomes in that same way but do not inhibit protein production in the remainder of the cell. The observed similarities have been offered as paleobiochemical evidence to revive and buttress the old notion (*9*) that organelles are derived fairly directly from prokaryotes which became endosymbionts of nucleated cells (*9*, *10*). To the extent that the endosymbiont hypothesis was imagined to be fact, the view that modern organelles should or might be as autonomous as their progenitors was accepted and expected. Attention has been focused on the nature of the endosymbiont but the problems of its domestication have been neglected.

Recently acquired information about relationships between the organelle and the nucleo-cytoplasmic system forces us to consider more seriously than before the possibility that eukaryotism arose not via endosymbiosis but by another route. It also requires that, regardless of whether or not we understand the mode of origin of eukaryotism, we must consider and seek the rules of intracellular evolution that have operated to bring the eukaryotic cell into its modern form since its origin more than 1×10^9 years ago (*11*). Organelle biology may now be reaching the stage where a few general principles will soon emerge.

Recent studies have shown that genes for some components of *Chlamydomonas reinhardi* ribosomes are in the nuclear genome and genes for others are in the plastid genome. In this article, this work is reviewed and its implications are considered with regard, first, to possible modes of origin of the eukaryotic system and, second, to possible mechanisms for intracellular-intergenomic gene dispersal. A number of other multimeric elements of plastids and mitochondria may also be the products of genes in more than one genome. In all of these cases is is necessary to resolve (i) mechanisms of gene dispersal as well as the factors that limit this process; (ii) possible devices by which nuclear gene-coded, cytoplasmically synthesized polypeptides may be locked into the organelle; and (iii) whether genes for mitochondrial proteins, for example, need be distributed between mitochondrial and nuclear genomes in the same way in all organisms. Each of these problems will be examined in turn.

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Location of Structural Genes for Organelle Components

Each animal mitochondrion contains too little DNA to code for all of its RNA's and proteins (*8*), but unequivocal evidence that at least one protein found in mitochondria is coded for by a nuclear gene came from the discovery that the structural gene for iso-1-cytochrome c of yeast is in the nuclear genome (*12*). This was the first identification of any gene coding for a component of any organelle. Nuclear genes that influence plastid development by affecting the concentrations of intracellular amino acids or iron had been identified earlier (*13*).

The ribosomal RNA's (rRNA's) of an organelle hybridize with its own DNA in vitro (*8, 14*). This shows that genes for the rRNA's are located in the organellar genome. However, chloroplast rRNA's hybridize with nuclear as well as chloroplast DNA in some plants (*14, 15*). There is no doubt that cistrons for organellar rRNA's are located in organellar DNA, although the possibility that some chloroplast rRNA cistrons are also present in the nuclear genome in some plants cannot be excluded. Organelles contain many distinctive tRNA's, that is, many transfer RNA's, different from those in cytoplasm. A number of these hybridize exclusively with organellar DNA (*8, 14, 16*). Thus, the DNA's of organelles contain genes for some unique tRNA's as well as for RNA's of the organelle's ribosomes. What else?

My colleagues and I (*17–21*) undertook to locate genes for proteins of chloroplast ribosomes among the genomes and linkage groups of the flagellated unicellular green alga *Chlamydomonas reinhardi*. Chloroplast ribosomes of *C. reinhardi* are 68*S* in size and dissociate into 52*S* and 37*S* subunits (*17, 18*). By two-dimensional electrophoresis in urea-sodium dodecyl sulfate polyacrylamide gels, 26 proteins have been distinguished in the 52*S* subunit, and 22 proteins have been distinguished in the 37*S* subunits (*19, 20*). The cytoplasmic ribosomes are distinctly different. The 81*S* cytoplasmic ribosomes dissociate into 61*S* and 41*S* subunits. The large cytoplasmic subunit contains 39 proteins; the small one contains 26 subunits. Only four pairs of proteins in the large subunits from the two kinds of ribosomes migrate similarly in the two-dimensional electrophoresis system. No more than these four proteins can be identical in the two subunits, but even these four may be different (*17–20*).

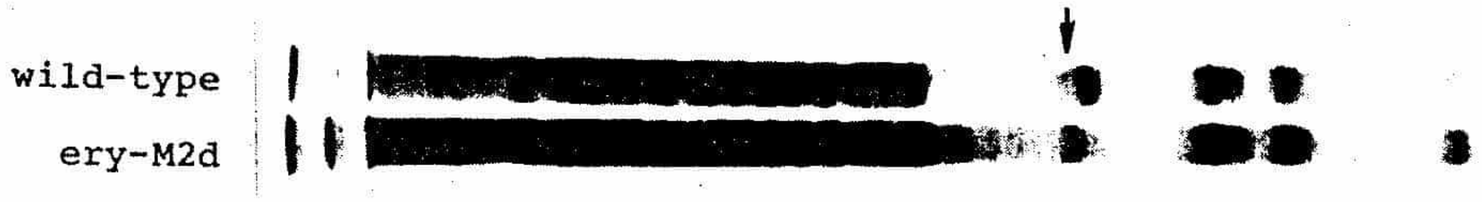


Fig. 1. Proteins of the large (52*S*) subunits of chloroplast ribosomes of wild-type (upper) and ery-M2d (lower) strains of *C. reinhardi* after electrophoresis on polyacrylamide gel in the presence of 8*M* urea (*18*). The arrow shows the position of the altered protein. Ribosomal proteins were obtained (*18*) by collecting and breaking *Chlamydomonas* cells; purifying ribosomes by centrifugation through a solution of buffered 1*M* sucrose; dissociating the ribosomal subunits and separating the four major types (large and small subunits of cytoplasmic and chloroplast ribosomes) from one another on a sucrose gradient; collecting the separated 52*S* subunits; and solubilizing the proteins by the LiCl-urea method of Leboy *et al.* (*53*). Plastid ribosomes make up about 30 percent of the total in *Chlamydomonas* preparations; mitochondria contain only a small fraction of the total ribosomes.

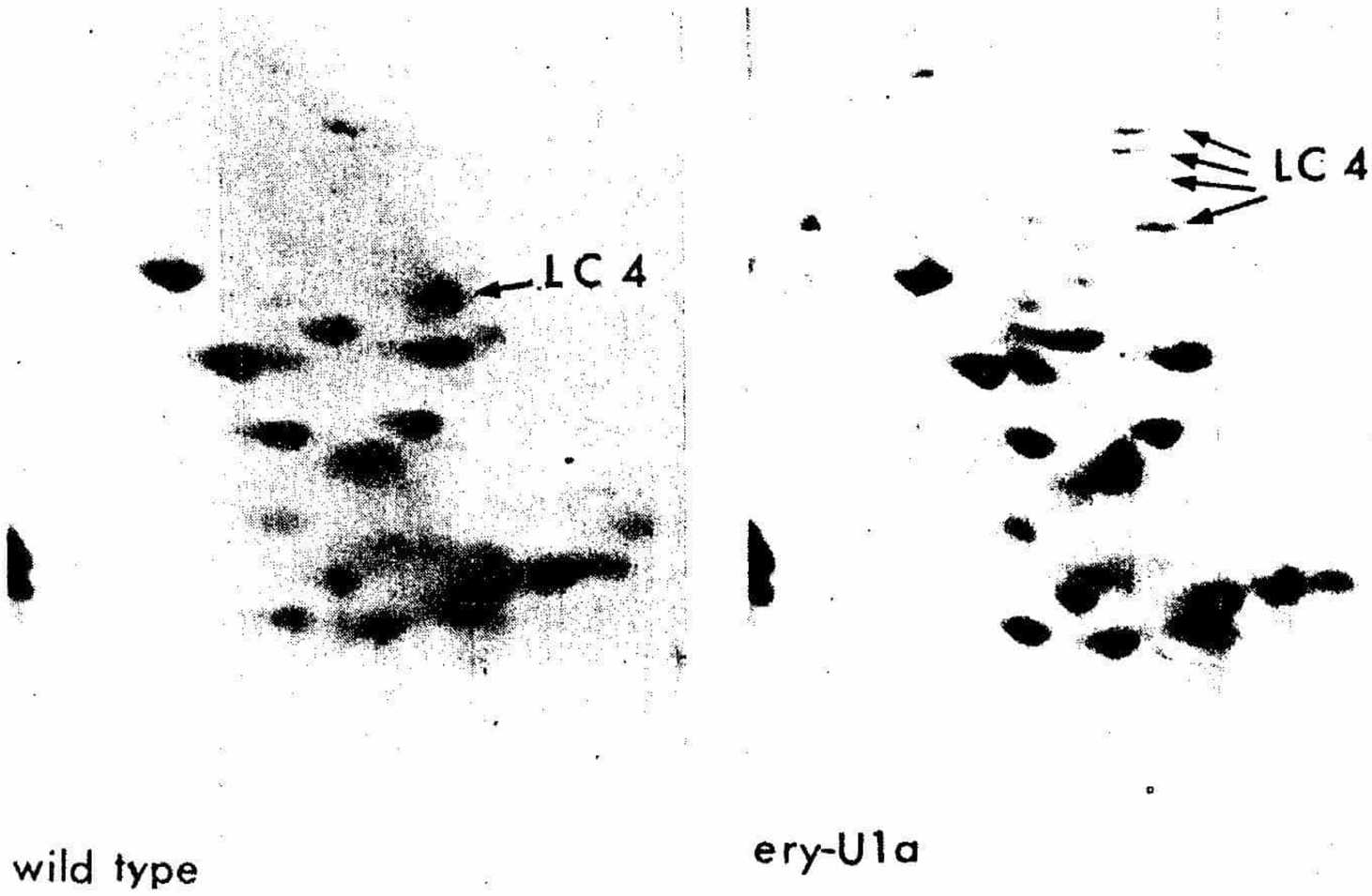


Fig. 2. Portions of two polyacrylamide gel slabs showing the results of electrophoresis in the presence of urea (left to right) and then in sodium dodecyl sulfate (top to bottom) proteins from the 52*S* subunits of *C. reinhardi* chloroplast ribosomes (*18, 19*). The slab on the left is a preparation from wild-type cells, that on the right is from cells of ery-U1a strain in which erythromycin resistance is transmitted uniparentally. The ery-U1 locus is in the extranuclear, chloroplast genome. Protein LC4 (*20*) is shown in the wild-type pattern and aggregated forms of this protein are indicated among the ery-U1 a 52*S* ribosomal proteins on the right.

The antibiotic erythromycin inhibits bacterial protein synthesis and kills bacteria and *Chlamydomonas*. It binds to the 52*S* subunit of chloroplast ribosomes but not to any other of the three subunits of ribosomes in the chloroplasts and cytoplasm of *C. reinhardi*. There is about one erythromycin binding site per 52*S* subunit; the equilibrium constant for binding is about $8 \times 10^4 M^{-1}$, that is, about one to two orders of magnitude below that for bacterial ribosomes (*17*). *Chlamydomonas reinhardi* strain 137c does not grow in liquid cultures containing more than about $1.4 \times 10^{-5} M$ erythromycin even when acetate is available. This alga can grow on acetate in darkness.

Chloroplast ribosomes of a group of erythromycin-resistant strains of *Chlamydomonas* failed to bind erythromycin (*17*). These mutants with altered plastid ribosomes have been used in genetic experiments to locate the genes for resistance and in biochemical experiments to determine the molecular basis of the resistance to this antibiotic (*17, 18, 20, 21*).

Chlamydomonas was used in these experiments in part because two of its genetic systems have been studied in detail. Characters in the nuclear genome are transmitted according to the well-known rules of Mendelian genetics. However, genetic determinants in an extranuclear chloroplast genome appear in all the progeny if introduced into the cross by the "plus" mating type parent, but they are lost if they are introduced in the "minus" mating type parent (*3, 22*). The erythromycin-resistant mutants were derived from wild-type strain 137c, mating type +. Therefore, crossing each of the mutants with "minus" mating type, erythromycin-sensitive wild-type cells would show that the gene is in the nuclear, Mendelian genome if half the offspring carried the resistance marker or such crossing would show that it resided in the uniparentally transmitted chloroplast genome if all of the offspring carried the marker. Vegetative cells of *Chlamydomonas* are haploid.

Genetic analyses showed that the genes for resistance are in the nuclear genome of eight of the strains and in the plastid genome in the ninth. The set of eight Mendelian mutants was subsequently judged to consist of two groups of four, which have been designated ery-M1 a, b, c, d and ery-M2 a, b, c, d (*17, 18*). The implication that each member of a group carries a mutation at the same locus has been verified for strains of the ery-M1 class (*21*). Thus the alterations that destroy the erythromycin binding site in the 52*S* subunit of *Chlamydomonas* plastid ribosomes could be associated with at least two genetic loci in the Mendelian genome and another locus in the uniparental genome. The molecular mechanisms for the failure to bind erythromycin remained to be determined.

Comparison of the proteins of the 52*S* chloroplast ribosome subunit from wild-type cells with those from ery-M2d cells revealed one with reduced electrophoretic mobility in the latter (Fig. 1). The mutation to erythromycin resistance assigned to locus ery-U1 in the extranuclear uniparental chloroplast genome has been correlated with an alteration in a second protein—identified as LC4, a 30,000-dalton protein of the 52*S* subunit (*18, 20*). Figure 2 shows identical portions of two-dimensional polyacrylamide gels of wild-type and ery-U1a 52*S* proteins. The mutation in ery-U1a results in the production of an aggregating form of protein LC4.

Mutants of the ery-M1 class have been studied more intensively. First, Davidson *et al.* (*21*) have determined that this locus is on linkage group XI of the *Chlamydomonas* nuclear genome [see map in (*3*)]. All four of the ery-M1 mutations map 11 to 15 units to the right of the marker "paralyzed flagellae 2" (pf2). It has been concluded that, within the limits of error of the measurements, the four mutations are at the same locus. If the ery-M1a-d mutations are allelic, the same protein should be aitered in all four mutants. This has proved to be the case (*21*).

Ribosomal protein LC6 in wild-type cells differs in net charge at *p*H 5 from LC6 in the ery-M1 mutants we have examined. Three distinguishably different forms of LC6 have been identified among four mutants that map to the locus ery-M1 (Fig. 3). The variety of altered forms of LC6 and the mapping of the genetic determinant to the same point on the same chromosome supports the view that the nuclear locus of erythromycin resistance of the ery-M1 group is the structural gene for chloroplast ribosomal protein LC6 of the 52*S* subunit (*21*).

This view is bolstered further by evidence of another type. Cells of a diploid

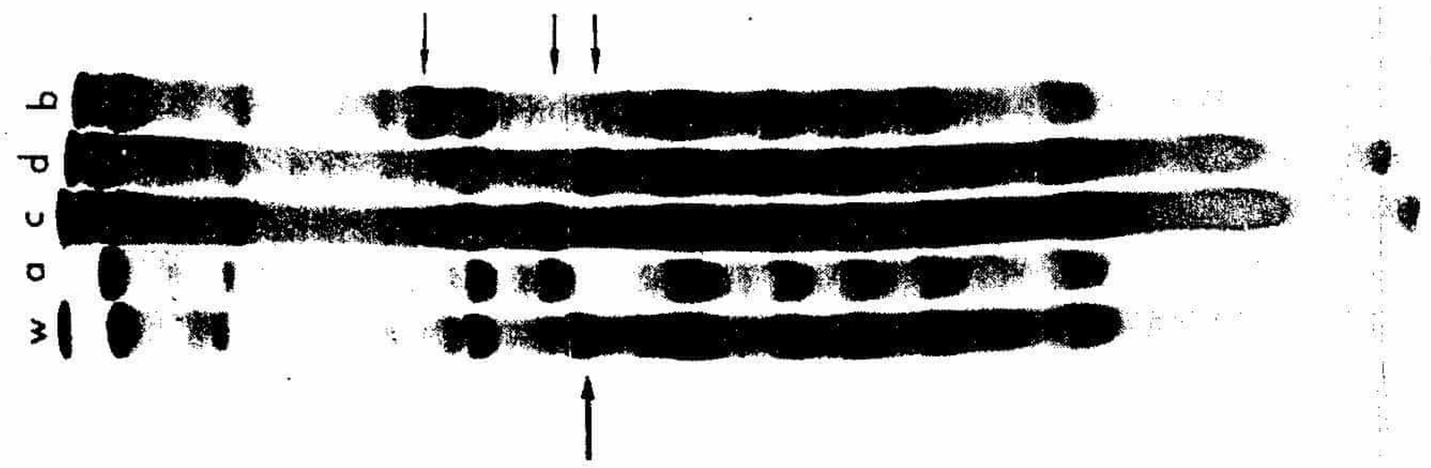


Fig. 3. Proteins of 52*S* subunits of *C. reinhardi* chloroplast ribosomes from wild-type cells (w) and of ery-M1 strains a, c, d, and b (bottom to top) after electrophoresis (from left to right) on polyacrylamide gels in the presence of 8*M* urea (*20*). The arrow on the bottom of the gels shows the position of the wild-type protein LC6 (*20*); the arrows on the top show the three positions to which altered forms of LC6 in the erythromycin-resistant mutants ery-M1a-d migrate. LC6 in ery-M1b is about one-third smaller than the wild-type form of this protein (*21*).

line of *Chlamydomonas* constructed by mating an erythromycin-sensitive strain (that is, wild type for ery-M1) with a strain bearing ery-M1c contain two forms of LC6: the wild type and that found in ery-M1c (*23*). Similarly, diploids between wild type and strains of ery-M1b or -M1d each contain both the normal and altered form of LC6 (*23*). If the alterations in LC6 in ery-M1 strains were manifestations of changes in the specificity of a protein-modifying enzyme, only one form of the ribosomal protein would have been present.

Ery-M1 is the first gene which has been identified for a chloroplast protein. It is also the first gene for a eukaryotic ribosomal protein that has been identified. Furthermore, these experiments demonstrate that genes for chloroplast ribosomal proteins of the 52*S* subunits in *Chlamydomonas* are scattered in three linkage groups through two genomes. Locus ery-M1 is not linked to the other nuclear gene ery-M2.

Gene Dispersal in Eukaryotic Genomes

The data showing that these three genes for chloroplast ribosomal proteins are scattered among three linkage groups in two genomes together with evidence from molecular hybridization experiments that identify genes for *C. reinhardi* chloroplast rRNA's in chloroplast DNA (*17–21, 24*) leave no doubt that these ribosomes are the products of two genomes and that genes for components of chloroplast ribosomes are separated. This forces us to consider possible mechanisms of intracellular gene dispersal, to weigh whether and why there may be limits to gene dispersal, and to attempt to discover the rules governing the evolution of eukaryotic genomes.

Before examining possible mechanisms for intracellular gene dispersal during evolution, we should recognize two possible modes of the origin of eukaryotic cells. One is the widely discussed (*9, 10*) origin by endosymbiosis. The outlines of this hypothesis have been presented above. Eukaryotism could also have originated by the cluster-clone route (*25*). According to the endosymbiont hypothesis two or three cells combined to make one. In the cluster-clone alternative a single cell is visualized to have been partitioned.

The establishment of the eukaryotic cell from an uncompartmentalized single cell according to the cluster-clone hypothesis could have proceeded somewhat as follows: (i) the formation of gene clusters (each cluster is a genome); (ii) the development of a membrane around each cluster of genes plus some protoplasm, to produce one or more gene-containing structures (from which nuclei, mitochondria, and chloroplasts would evolve) and the gene-free space, that is, the cytoplasm; and (iii) the division and faithful reproduction of each gene-containing compartment to give rise to clones of nuclei, chloroplasts, and mitochondria which, together with the cytoplasm, have evolved to the many forms of eukaryotic cells now extant.

A stepwise variant on this pattern is also easy to imagine. For example, a first step would be the formation of the nucleus as outlined. The detachment of groups of genes as plasmids that become established in membrane-limited forms outside of the nucleus would be a second step. This sort of sequence has been suggested by Raff and Mahler (*26*).

Examination of possible mechanisms for ribosomal gene dispersal is simpler if the initial discussion is limited to the origin of eukaryotism by endosymbiosis and ribosomes are assumed to have originated only once. According to the endosymbiont hypothesis both the nucleated and anucleate cells which joined would have evolved at some earlier point from a common ancestral type containing one kind of ribosome. In the course of further separate evolution of the two types of cells, ribosomes with some distinctive properties developed. Two

or three types of ribosomes would have been brought into the same cell when endosymbiosis began; one type of ribosome with each "invader." Thus, at that time all of the genes for the ribosomes of each endosymbiont would have been in it along with the ribosomes themselves. In each case the genes would have been together with the gene product. Today, at least in the case of *Chlamydomonas* chloroplast ribosomes, the genes and the gene products are separated in different compartments.

One way in which organellar ribosomal protein genes could have dispersed is by a gene transfer mechanism (Fig. 4). Nonsexual gene transfer and integration into the chromosome or into the hereditary mechanism is experimentally verified in bacteria. The integration of viral genes into the nuclear genome of mammalian cells is an established phenomenon. The shifting of genes from one genome to another within a eukaryotic cell seems a reasonable possibility. As illustrated in Fig. 4, if an organellar gene duplicates and a copy is intercalated into the nuclear genome, the gene is represented twice. If the organelle gene is subsequently lost, the gene for the organelle component is solely in the nuclear genome. The nature of pressures for selective loss are not known. Transfer of a gene without prior duplication is equally likely.

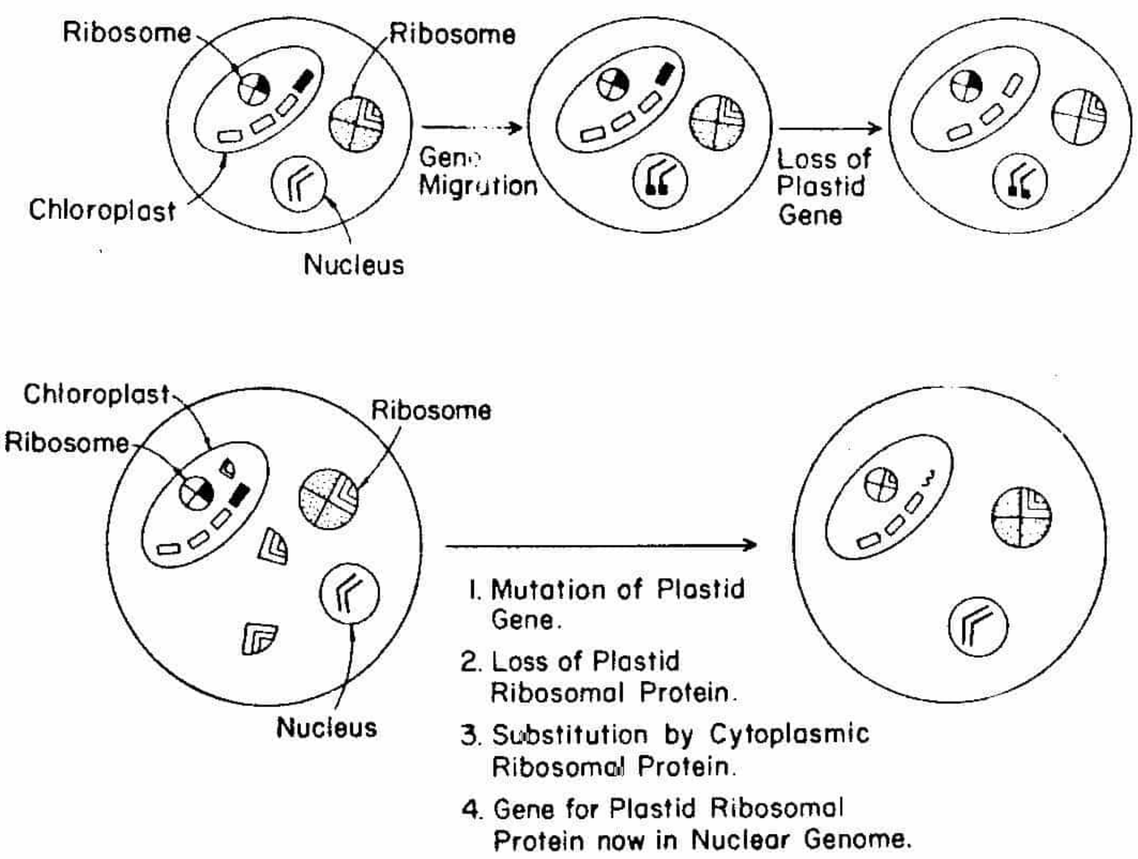
Dispersal by protein and gene substitution (Fig. 5) is more complicated. If an organellar gene mutated to specify a protein useless for the organelle ribosome, then the ribosome, the organelle, and perhaps the cell could be rescued if another protein in the cell could substitute—even a poor substitute could be adequate. If the substitute protein were coded for by a nuclear gene, the cytoplasmically synthesized protein and its nuclear gene would take the places of the organellar protein and its gene. A further series of gene duplications and mutations could lead to the substitute protein and substitute gene serving the organelle alone.

More generally, if any of the proteins brought in by the endosymbiont were functionally interchangeable with those of the host, some organelle genes might be retained to provide information for cytoplasmic components; nuclear genes might be retained for organelle components. Selections in these two directions may differ from one evolutionary line to another. Internal or external selective pressures for the retention of a particular gene in the nucleus or organelle have yet to be identified.

For simplicity, evolution after the creation of eukaryotism via endosymbiosis has been discussed first. However, the mechanisms of gene reduction and dispersal already proposed could be the same during evolution after the establishment of eukaryotism via either endosymbiosis or the cluster-clone route.

The cluster-clone hypothesis begins with the proposition that genes in the prokaryotic ancestor become associated into clusters; for example, the genes are together in one chromosome which breaks up and each part segregates or, in a multichromosomal organism, one or some chromosomes separate from the others, or the genes are relatively "loose" and cluster. The pattern of clustering would also depend on whether one or more than one copy of each gene is present in the organism. The one copy of each gene case is illustrated in Fig. 6a. Each cluster would have to contain a unique set of genes; this is the way genes for some ribosomal components could be in one cluster and genes for other components in a different cluster from the very beginning of eukaryotism. This resembles the situation that exists today. If two or more copies of each gene were present in the prokaryotic ancestor, one extreme clustering pattern might be represented by Fig. 6b. Each gene is present in every cluster, much like the modern situation for the nuclear genome in multinucleate cells and for organelle gene distribution in organisms with more than one mitochondrion or plastid (or both) per cell. Evolution from this starting condition has many features in common with endosymbiosis except that here the present gene distribution could be achieved by selective gene reduction alone.

Uzzell and Spolsky (*27*) have suggested a program not unlike that shown in Fig. 6b. Meyer (*28*) has proposed a single-copy type of cluster-clone origin of mitochondria: One cluster is a plasmid containing genes for ribosomal proteins, rRNA's, tRNA's, respiratory enzymes, and the like, which became associated with the membrane; the description suggests that perhaps the remainder of the bacterial genome comprises the other cluster, yet the plan is said to offer "the interesting possibility of harboring a master copy of the mitochondrial genome in the nucleus. . . ." Perhaps Meyer is suggesting a cluster-clone plan that starts in a cell having a single copy of some genes and two copies of others. The set of duplicates is both in the main chromosome and in the plasmid of the prokaryote. Finally, Nass (*29*) has proposed a plan somewhere between endosymbiosis and a cluster-clone progression. The members of a colony of prokaryotes acquire different specialized function by selective loss and later "there might be a communication network between the com-



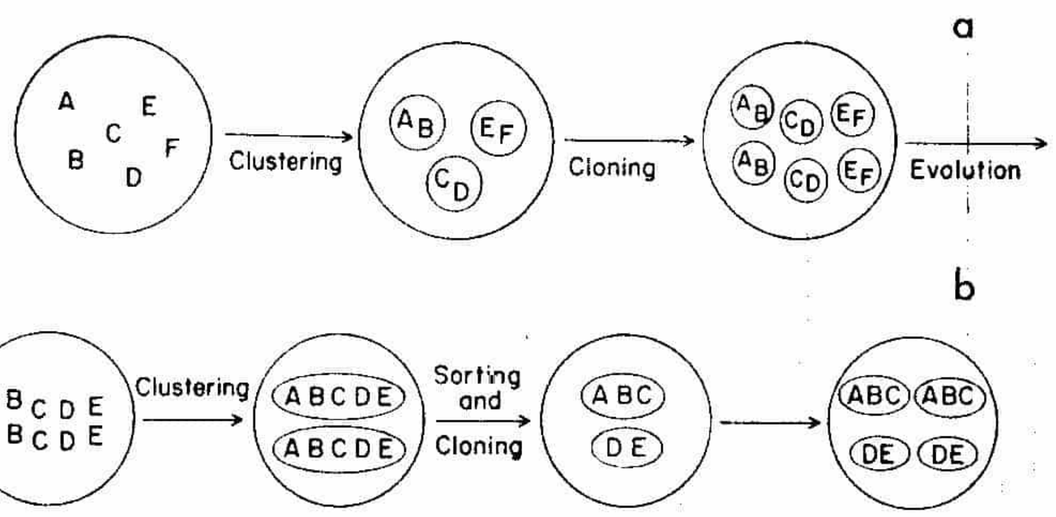


Fig. 4 (upper left). A schematic representation of gene dispersal by transfer. The rectangles in the chloroplast represent genes. The "solid" gene codes for the "solid" chloroplast ribosomal protein in this illustration.
Fig. 5 (lower left). Representation of gene dispersal by protein and gene substitution. A cytoplasmic ribosomal protein is shown to substitute for the defective ribosomal protein of the organelle only as an illustrative possibility. Fig. 6 (above). Schematic diagrams of the cluster-clone hypothesis of the origin of eukaryotic cells. (a) In this case only one copy of each gene is present in the prokaryotic cell. (b) This is one of a large number of possible patterns of gene clustering, organelle cloning, and gene sorting during subsequent evolution starting from a prokaryotic cell in which more than one copy of every gene is present.

ponents." The plan suggested by Nass would also require some intracellular gene shifting to account for present distributions of some genes and their products.

Shifts of genes among plastid, mitochondrial, and nuclear genomes may still be occurring although the frequency of displacements is likely to be lower in a stable species than at the time of the origin of eukaryotism. As more structural genes are located, perturbations of the steady state may permit experimental verification of the proposals made here and elucidation of the forces that promote or limit intracellular dispersal of genes.

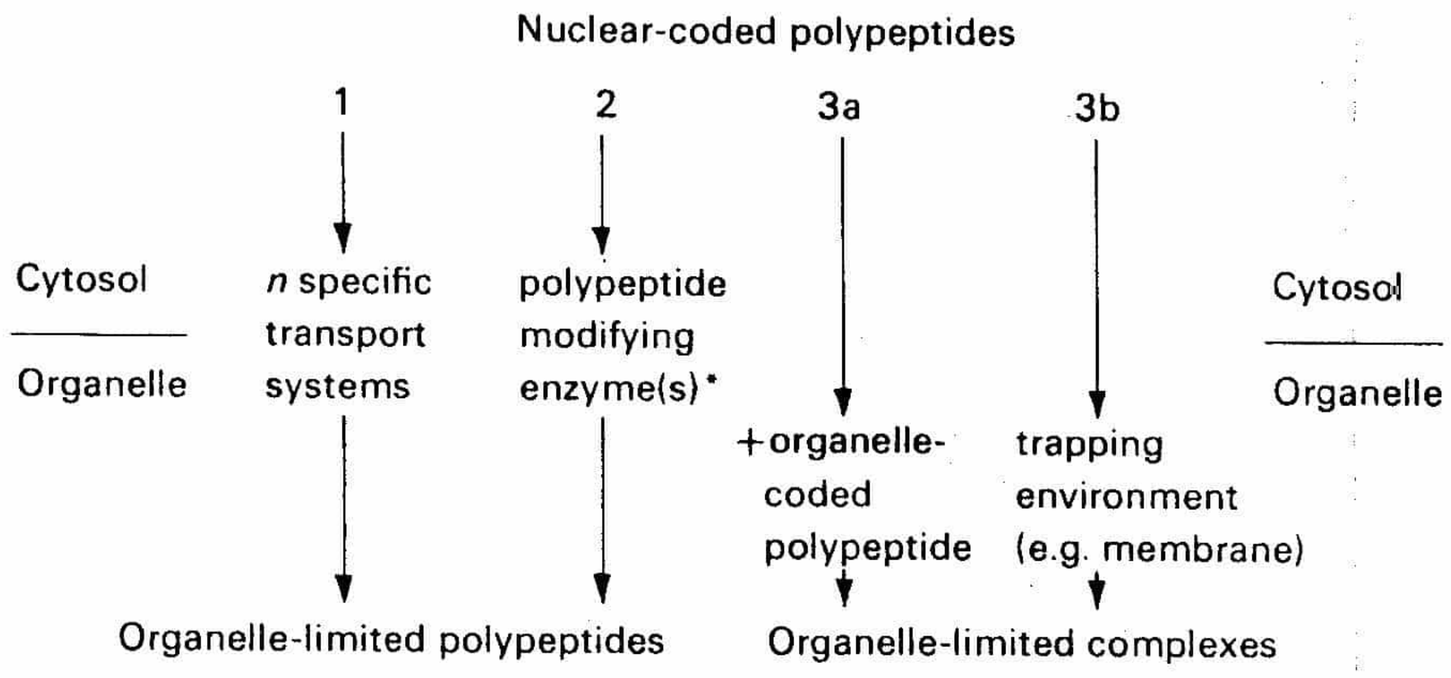


Fig. 7. Possible lock-in mechanisms for polypeptides coded by nuclear genes.

Intergenomic Cooperation as a Principle of Organelle Biology

Apart from *Chlamydomonas* chloroplast ribosomes there are other cases of gene dispersal. Genes for proteins of complex organellar elements such as multimeric enzymes and membranes may also be dispersed in two genomes.

In one group of cases, there is direct evidence to assign the genes that either specify or modify the protein to the nuclear or organellar genome:

1) The locations of genes for rRNA's and some proteins of *Chlamydomonas* plastid ribosomes has already been discussed. The structural gene for LC6 has been identified as locus ery-M1 in the nuclear genome; mutations at loci ery-M2 and ery-U1 result in alterations of specific ribosomal proteins of the 52*S* subunit. The rRNA of chloroplast ribosomes hybridize with chloroplast DNA.

2) The acrylamide gel electrophoretic patterns of solubilized chloroplast membrane proteins of *Acetabularia calyculus* and *A. mediterranea* are alike except for differences in three sections of the gel (*30*). Six weeks after isolated nuclei of *A. mediterranea* were transferred into basal parts of anucleate *A. calyculus* the gel pattern exhibited by the proteins resembled that of *A. mediterranea*, the source of the nucleus. The converse results were also obtained by Apel and Schweiger (*30*) when isolated nuclei of *A. calyculus* were implanted into basal segments of nucleated *A. mediterranea*. The electrophoretic migration of these proteins could change because products of genes in the introduced nucleus bring about secondary alterations in organelle-coded membrane proteins. However, it is at least as likely that these data show that genes for some chloroplast membrane proteins in *Acetabularia* are in the nuclear genome. Others may be in the plastid genome.

3) Kloppstech and Schweiger (*31*), using transplantation techniques similar to those described above, have shown that some chloroplast ribosomal proteins behave differently on electrophoresis depending upon whether the homologous or heterologous *Acetabularia* nucleus is introduced into an enucleated cell.

4) Ribulose-1,5-diphosphate carboxylase of green plants consists of two subunits. One is about 14,000 and the other about 50,000 daltons. Wildman and his collaborators (*32*) have studied the patterns of inheritance of the two subunits of this enzyme in crosses of species of *Nicotiana*. This is the sexual cross equivalent of the *Acetabularia* nuclear transplantation experiments; in both cases advantage was taken of natural variations in a given protein in related species. The small subunits of the ribulose-1,5-diphosphate carboxylase from *N. glauca* and *N. tabaccum* differ in tyrosine content and in the patterns of tryptic peptides. Differences in chromatographic or electrophoretic behavior of tryptic peptides could be due to secondary modifications of the proteins, but the demonstration of differences in gross amino acid composition of the small subunits of ribulose-1,5-diphosphate carboxylase from the two species increases the probability that primary differences in sequence are being revealed in the peptide maps. Information for the small subunit appears to be transmitted in a Mendelian manner in crosses between *N. tabaccum* and *N. glauca*. In addition, synthesis of the *N. glauca* type of small subunit is depressed in the progeny of this cross.

The tryptic peptide map of the large subunit of ribulose-1,5-diphosphate carboxylase from *N. gossei*, an Australian *Nicotiana*, has one more peptide than the corresponding protein from *N. tabaccum*. The extra peptide appeared in reciprocal F_1 hybrids only when *N. gossei* was the female parent. The amino acid compositions of the large subunits from the two species are not reported, and thus the evidence for inheritance of different primary amino acid sequences is not quite as strong as for the small subunit.

5) There are two electrophoretically detectable differences between proteins of the large subunits of chloroplast ribosomes of *N. tabaccum* and *N. glauca*. The differences are transmitted in a Mendelian manner (*33*).

In another group of cases, evidence for the location of the genes is even less direct than any of the above and also requires the unproven assumptions that cytoplasmic ribosomes only translate nuclear messages and that an organelle's ribosomes only process messages transcribed from its DNA. Through judicious and circumspect use of chloramphenicol and cycloheximide, antibiotics which at least in vitro specifically inhibit the functioning of organelle or cytoplasmic ribosomes respectively, it has been shown that some polypeptides of *Chlamydomonas* chloroplast membranes are formed on chloramphenicol-sensitive organellar ribosomes and others on cycloheximide-sensitive cytoplasmic ribosomes (*34*). Some subunits of mitochondrial adenosine triphosphatase in yeast are formed by mitochondrial and others by cytoplasmic ribosomes (*35*). Cytochrome oxidase is a multimeric membrane-bound enzyme in mitochondria. Some subunits are made on mitochondrial ribosomes and others on cytoplasmic ribosomes (*36*). Similar analyses indicated that the formation of the small subunit of ribulose-1,5-diphosphate carboxylase is preferentially blocked by cycloheximide and that chloramphenicol inhibits the formation of the large subunit (*37*).

Using a third approach, Blair and Ellis (*38*) have shown that isolated chloroplasts of peas can synthesize or complete the synthesis of the large subunit of ribulose-1,5-diphosphate carboxylase. It has been confirmed by other techniques that one subunit of the carboxylase is made on chloroplast ribosomes and the other on the larger ribosomes of the cytoplasm (*39*).

The phenomenon of intracellular-intergenomic gene dispersal is established by the studies on the location of genes for *Chlamydomonas* chloroplast ribosomal proteins and rRNA's. Other data can be interpreted to indicate that the separation of genes for complex organellar elements may be a general principle of organelle and eukaryote biology. For example, (i) if all the cases in which nuclear genes have been shown to influence the electrophoretic behavior of organelle polypeptides are assumed to indicate that the structural genes for these proteins eventually will be traced to loci in the nuclear genome, (ii) if cytoplasmically synthesized proteins are assumed to all be made only from messages transcribed from nuclear genes, and (iii) if organelle ribosomes only translate messages transcribed from organelle genes, then the dispersal of genes for components of multimeric organellar structures in two genomes is certainly a common phenomenon and perhaps a principle. Separation among three genomes may be common in cells that contain plastids and mitochondria.

It seems reasonable to assume that, as in the case of genes for chloroplast ribosomal proteins and rRNA's, the genes for the various polypeptides of each structure were together and in the same compartment as the gene product just before or just after the establishment of the eukaryotic habit by way of the cluster-clone or endosymbiont paths, respectively. The possible gene dispersal mechanisms already suggested in the ribosome case could apply equally to any of these.

Limits to Gene Dispersal

How far can gene dispersal go (*40*)? Or, to state the question at its extreme: Why aren't the genes for all components of a mitochondrial or plastid-limited multimeric element in the nuclear genome?

The analysis in the immediately preceding section reveals that there may be some limits to gene dispersal. In principle, a single protein or nucleic acid contained within a membrane should be sufficient to specify an entire organelle, but there are no mitochondria or plastids in which this is known to occur.

A component may be coded for and produced within the organelle because it is untransportable—that is, mechanisms for its transport could not be evolved fast enough if the gene were transferred to the nuclear genome—and there is no substitute available. On the other hand, the gene might be retained because the product is necessary for locking nuclear-coded cytoplasmically synthesized polypeptides into the organelle. There must be some devices for partitioning a protein into an organelle and keeping it there. Figure 7 represents an attempt to summarize some possible devices for locking nuclear-coded cytoplasmically synthesized proteins into an organelle.

Specific transport devices (lock-in device 1). A specific transport system for each polypeptide would be possible but seems incredible. In contrast, if each protein which comes to be localized in the organelle contains an amino acid sequence that identifies it for a transport system, this possibility becomes more credible; fewer transport systems would be necessary. A device of this sort seems entirely reasonable for organelle-limited soluble proteins. It could also operate to take cytoplasmically synthesized subunits of multimeric enzymes into mitochondria (see *41*).

Linear arrays of cytoplasmic ribosomes have been seen aligned along the outsides of, for example, yeast mitochondria (*42*) and maize plastids (*5*) and have remained associated with purified organelles (*43*). Kellems *et al.* (*42*) found that about half of the puromycin-discharged nascent polypeptides from cytoplasmic polysomes attached to yeast mitochondria remained associated with the outer mitochondrial membranes. If this association is not an artifact and if the nascent polypeptides are not membrane components, these data may point to details of at least one type of lock-in device 1.

Protein modifying enzymes (lock-in device 2). A modification of a polypeptide which commits it to accumulating and operating in the mitochondrion or chloroplast is exemplified by *Neurospora* mitochondrial leucyl-tRNA synthetase (*44*). Both the cytoplasmic and mitochondrial leucyl-tRNA synthetases appear to be specified by the same nuclear gene, but the enzymes themselves are distinctively different; the modifying enzyme has not been identified. A modification could improve the transportability of the protein into the organelle.

Complexing of a nuclear-coded polypeptide with an organelle-coded component to form an organelle-limited complex (lock-in device 3a). The assembly of chloroplast ribosomes in *Chlamydomonas* and of ribulose-1,5-diphosphate carboxylase in *Nicotiana* are two examples.

A nuclear-coded polypeptide fits into a trapping environment (lock-in device 3b). An example of lock-in device 3b is an organelle membrane. This situation is illustrated (i) by the assembly of photosynthetic membranes in *Acetabularia* chloroplasts and (ii), if the site of synthesis of the polypeptide reflects the location of the gene, by the formation of photosynthetic membranes in *Chlamydomonas* chloroplasts. In a much more general sense, differences in, for example, ion concentrations in the cytoplasm and the interior of the organelle could affect some proteins to trap and lock them into the plastid or mitochondrion.

Can any systematic relationship be deduced between the possible lock-in devices and the proposed intracellular gene dispersal mechanisms?

Lock-in device 1 would require concommitant evolution of nuclear-coded organelle-limited polypeptides and of organelle membrane transport systems. Lock-in device 2 involves the secondary modification of a polypeptide chain. Changes in proteins for lock-in devices 1 and 2 might be expected if gene dispersal occurred by protein and gene substitution. The evolution of primary or secondary changes would be to improve the uptake or utility of a protein which was satisfactory at the initial substitution time. However, the polypeptide modifying enzyme of lock-in device 2 could act to improve the transportability of a protein whose gene was shifted from the organelle to the nucleus; lock-in device 2 is not incompatible with either of the two patterns of gene dispersal.

It is implied in lock-in devices 3a and 3b that the nuclear-coded polypeptide bears a recognition site for a place in the organelle-limited complex. Such a site would be retained in the original polypeptide if gene dispersal were by transfer. However, a protein could have served as a substitute only if some of its characteristics permitted it to fit into the position occupied by the original polypeptide at the outset; it could evolve to be better. Thus, devices 3a and 3b would be compatible with gene dispersal through either gene transfer or protein and gene substitution.

I have discussed the matter of why any genes are retained in the organellar genome. However, if we accept previously stated assumptions, genes not for one but for several components of *Chlamydomonas* chloroplast ribosomes, *Acetabularia* and *Chlamydomonas* chloroplast membranes, and even the simpler cytochrome oxidase and adenosine triphosphatase of yeast mitochondria are likely to be in the organelle. Two reasons are attractive: (i) again, some components may not "travel well"; and (ii) according to specifications of lock-in devices 3a and 3b the organelle-synthesized components of the complex are needed for anchoring—two anchors give more insurance than one.

This attempt to catalog and systematize possible mechanisms for gene dispersal and devices for nuclear gene product lock-

in was undertaken as a start toward determining some general principles of organelle biology. (i) It has been proved in only two or three cases, but it may always be the case, that some genes for parts of complex organelle structures are in the organelle genome; other genes are in the nuclear genome. This genomic interdependence is probably important in intracellular integration, that is, in the coordination of metabolism and growth of organelles and the nucleo-cytoplasmic system. (ii) The minimum number of genes within the organelle may be related to the necessity for locking the cytoplasmically synthesized components of the complex into the organelle and to insuring that at least one anchor remains in the organelle. (iii) An organelle with a few large complexes, such as ribosomes and an energy transducing membrane system, should require a smaller umber of genes in its genome than an organelle with more separate components, whether simple or complex. The number of genes to be sequestered must obviously also be greater if the organelle can develop in a number of different directions, such as a plastid, than if it can take only one form.

Natural Diversity in Intracellular Gene Distribution

Genes that specify proteins of the organelle are likely to be distributed differently among the nuclear, mitochondrial, and plastid genomes of the cell in different taxonomic groups. Even if eukaryotism originated only once, about 1.3×10^9 to 1.7×10^9 years ago (*11*), there has been ample time for the organellar and nuclear genomes to exchange or substitute components differently in each genetically isolated group of organisms. In addition, Raven (*45*) has examined evidence for the possible polyphyletic origin of chloroplasts among algal groups, assuming an endosymbiotic origin of organelles.

There is evidence that such variation may exist. For example, although mitochondrial functions are not known to differ, the DNA per mitochondrial genome varies from about 30 μm in peas (*46*) to 25 μm in yeast (*47*); 19 μm circles are present in *Neurospora crassa* mitochondria (*48*); 17.6 μm linear strands have been found in mitochondria of *Tetrahymena pyriformis* (*49*); DNA circles ranging from 4.45 to 5.85 μm in circumference constitute the mitochondrial DNA of various animals [for example, table VI in Borst and Kroon (*50*)]. These differences, even among mitochondria of higher animals, appear to be real (*51*). Determinations of kinetic complexity show that even the largest mitochondrial DNA's have very few redundant sequences. The larger mitochondrial DNA's do not simply have many more copies of the same number of genes present in smaller mitochondrial DNA's; additional different genes are most likely present although information content is not necessarily directly proportional to molecular weight despite an absence of repetitive sequences.

Among social insects the unit of selection and evolution is the community and the tasks within this community can be divided among the individuals in a variety of ways (*52*). All of them are successful. So, in the case of eukaryotic cells, where the unit of selection is the cell, a variety of successful solutions to the problem of subdivision of function should be expected. It seems unlikely or at least not obligatory that a gene for a specific organelle component will always be in the nuclear or always in the organelle genome in every species unless some rule like a preferential site of synthesis of certain classes of proteins (for example, those which are hard to move through the cell) sets a lower limit.

Summary and Conclusions

The structural gene for *Chlamydomonas* chloroplast ribosomal protein LC6 is on linkage group XI in the nuclear genome. Another nuclear gene affects a different protein of the large subunit of the chloroplast ribosome and the mutation of an extranuclear gene results in a change in chloroplast ribosomal protein LC4. Genes for plastid rRNA's are found in their DNA's. This is one clear case of the dispersal in two genomes of structural genes for components of a single organelle complex. The evidence that one subunit of ribulose-1,5-diphosphate carboxylase is coded by a Mendelian gene and the other by a maternally transmitted gene is also good. If some as yet unproved assumptions are made, there are several other cases of intergenomic gene dispersal. This may be a principle of organelle and eukaryotic biology. The questions of how the present gene distribution came about, what limits it, and whether the distribution patterns are universal among eukaryotes still have not been completely resolved. Some hypotheses have been advanced in this article.

All the genes for a structure as well as their products were together when or just before a eukaryotic cell formed. The genes are now scattered; the genes and their products are in different cell compartments. "Gene transfer" and "gene and protein substitution" are suggested as two possible mechanisms of intracellular gene rearrangements. If eukaryotic cells originated by one kind of cluster-cloning, the genes could have separated at the time eukaryotic cells originated.

Some genes for a complex may have to remain within the organelle to provide an anchor for the nuclear gene-coded cytoplasmically synthesized peptides although other types of lock-in devices are possible (Fig. 7). If the anchoring view is correct, we might conclude that the more the organelle's components are consolidated into a single structure, such as membrane, the fewer the number of genes that must remain in the organelle's genome to insure a complete plastid or mitochondrion.

Finally, there is no reason to believe that chloroplasts of all species or strains contain genes for the same organelle or cytoplasmic components or that mitochondria are so uniform. Some evidence already available suggests the contrary. Thus, not only is the nutrition of organelles very complex—special proteins are required—but the nutritional requirements are likely to vary greatly.

This discussion has emphasized the dynamic nature of the genetic relationships between organelles and the nuclear-cytoplasmic system in eukaryotic cells. Not only may we find a different distribution of genes among genomes of eukaryotic organisms, but the distribution itself is most probably not permanently fixed in all individuals of a species. Gene distribution "mutants" are likely to occur.

The models examined here provide possibilities for experiments that may elucidate principles of genomic interaction and intergenomic cooperation. The search for principles of intracellular gene distribution is one proper goal of current studies in organelle biology.

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